Research objective

Overarching question: Which nutrients are controlling primary production and when?

1. Nutrient Limitation and HAB Primary Production

Determine the potential for N, P, and/or N+P limitations to influence the primary production/growth of algal and cyanobacterial species.

2. Seasonal Nutrient Limitations of HAB Primary Production

Determine if there is a seasonal component (i.e., spring, summer, and fall) driving the potential nutrient limitations and algal and cyanobacteria species growth.

3. Spatial Nutrient Limitations of HABs

Determine whether there is a spatial aspect to the nutrient limitations of algal or cyanobacterial growth

(i.e., Provo Bay; main body of lake, east; main body of lake, west).

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Nitrate, ammonium, and phosphorus drive seasonal nutrient limitation of chlorophytes, cyanobacteria, and diatoms in a hyper-eutrophic reservoir

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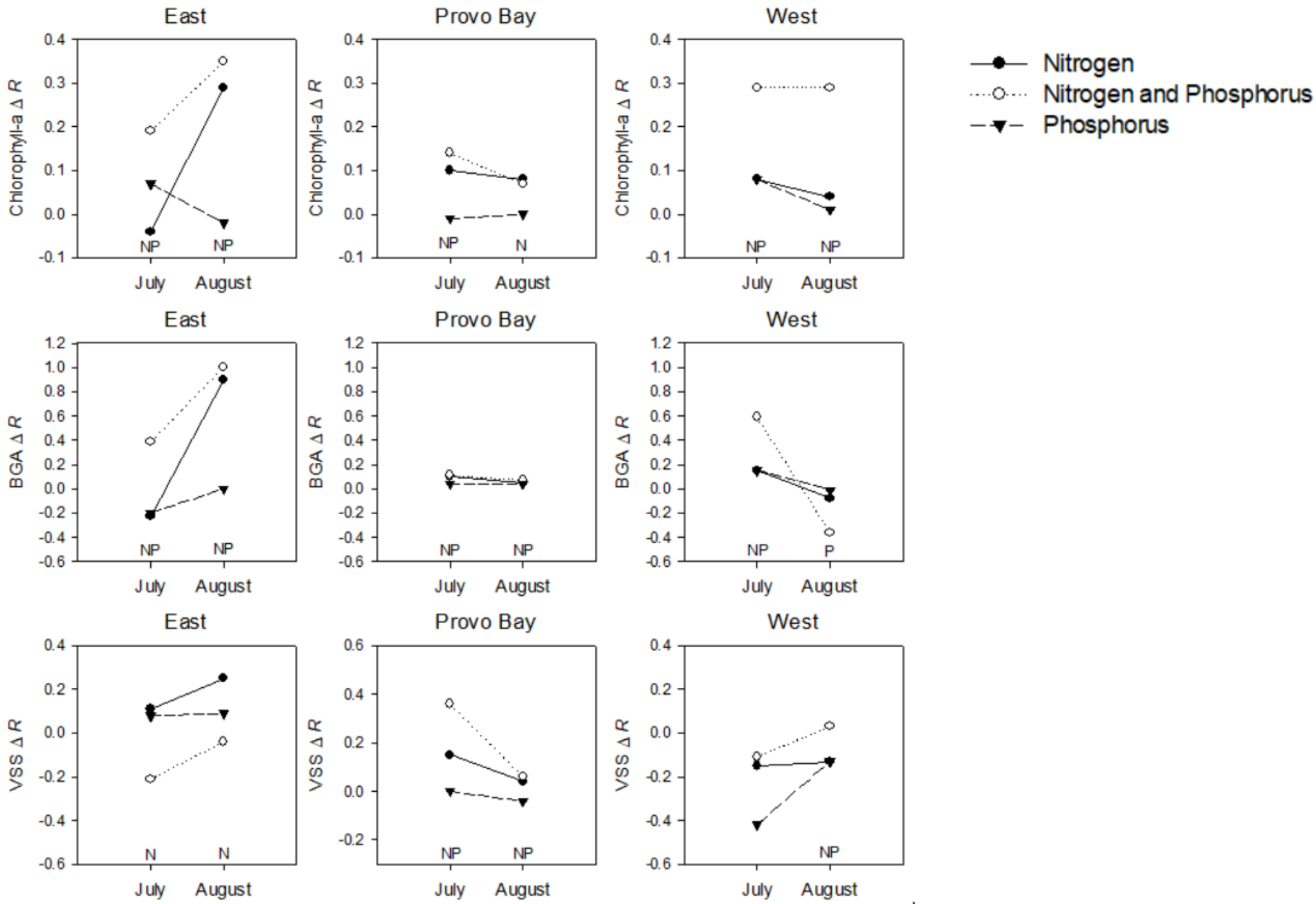
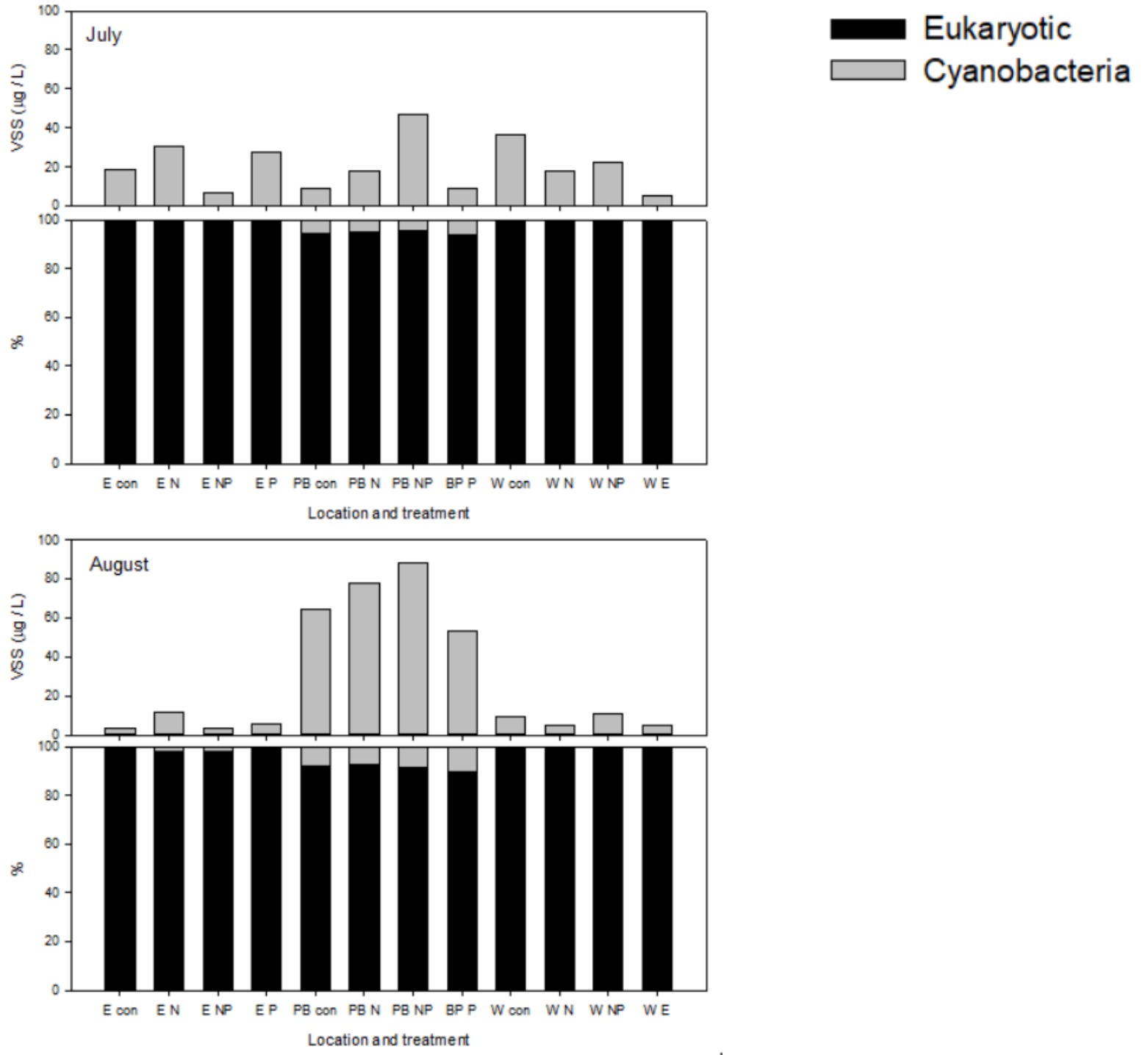
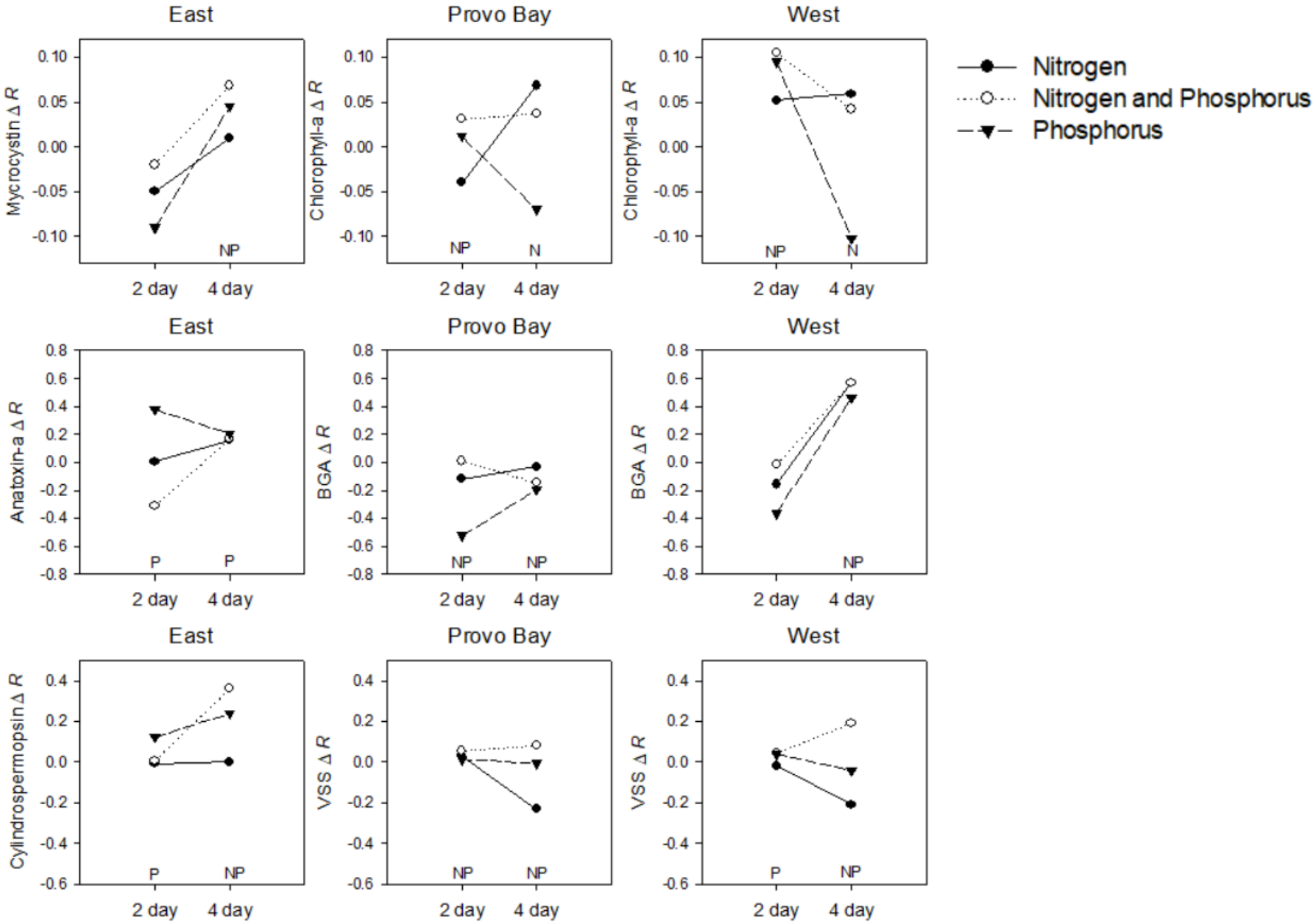
We completed We quantified phytoplankton nutrient limitation and response to different N forms as the growth response (ΔR) during the 48 h incubation period. Growth response was calculated for each taxonomic group as:

$$\Delta R = \log10 (\text{avg chl treatment} / \text{avg chl control})/t$$


East
Provo Bay
West
Chlorophyll-a Δ R
BGA Δ R
VSS Δ R
July
August
NP
N
P
Nitrogen
Nitrogen and Phosphorus
Phosphorus

Measurement	Completed for bioassays (summer = S, late summer = LS, and fall = F
Water chemistry sonde: pH, temperature, electrical conductivity, dissolved oxygen, turbidity, dissolved organic matter, blue green algae-phycocyanin *, and chlorophyll-a *	S, LS, F
Chlorophyll-a (ethanol extraction) *	S, LS
ELISA toxins (microcystin, anatoxin-a, and cylindrospermopsin)	S, LS
TSS and VSS to estimate photosynthetic biomass *	S, LS, F
TP and TN SRP, and ammonium and nitrate	S, LS
Cyanobacterial species and algal division composition direct microscopy *	S, almost all LS
RNA transcript extractions	started S
RT-qPCR cyanobacteria biomass *	-
RT-qPCR of nifH to estimate biological nitrogen fixation	-
Sequencing of cyanobacterial composition	-


July
August
VSS (ug / L)
%
Location and treatment
E con
E N
E NP
E P
PB con
PB N
PB NP
BP P
W con
W N
W NP
W E
Eukaryotic
Cyanobacteria


East
Provo Bay
West
Mycrocystin Δ R
Chlorophyll-a Δ R
Chlorophyll-a Δ R
Anatoxin-a Δ R
BGA Δ R
BGA Δ R
Cylindrospermopsin Δ R
VSS Δ R
VSS Δ R
0.10
0.05
0.00
-0.05
-0.10
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
NP
N
P
2 day
4 day
Nitrogen
Nitrogen and Phosphorus
Phosphorus

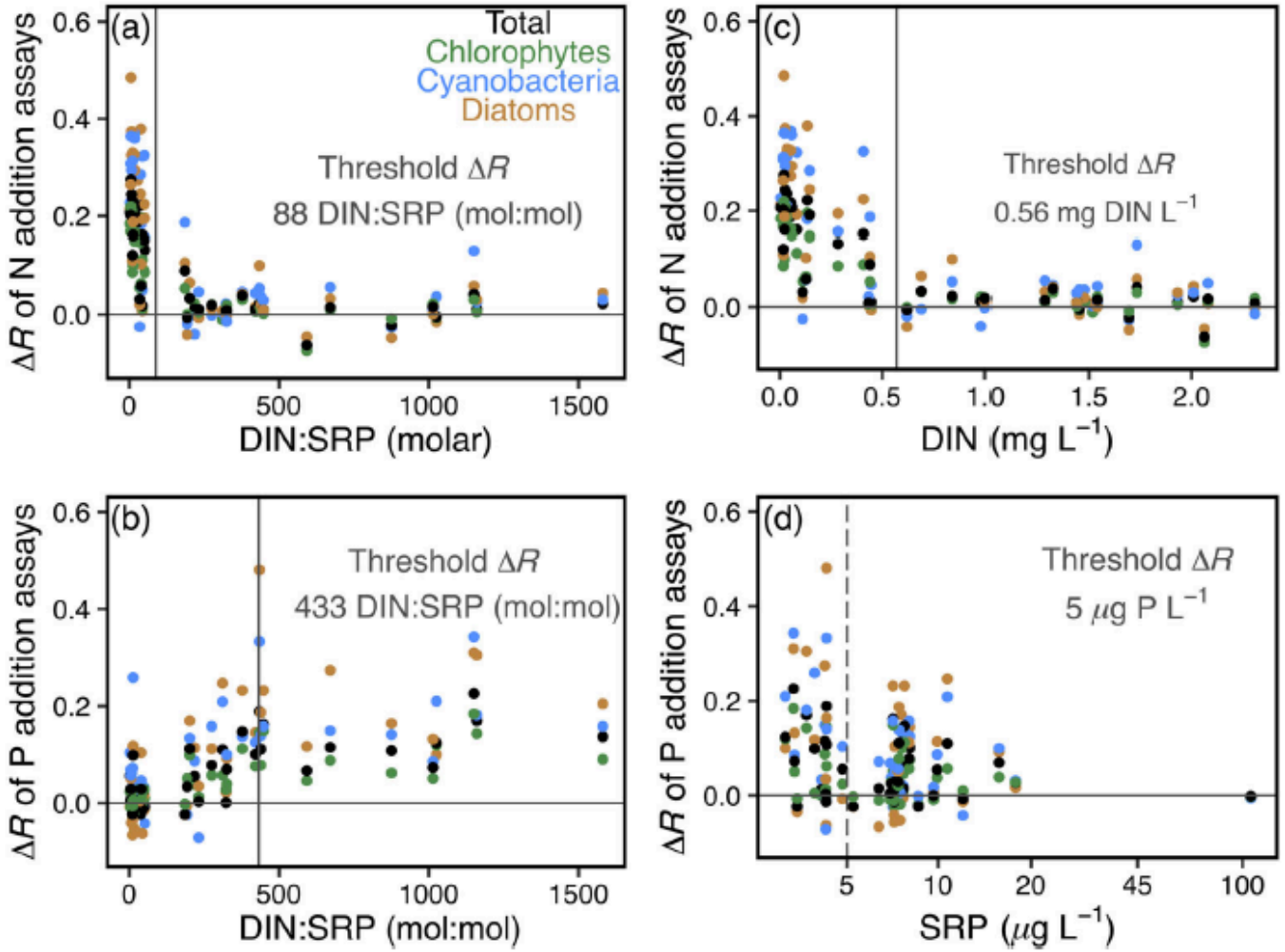
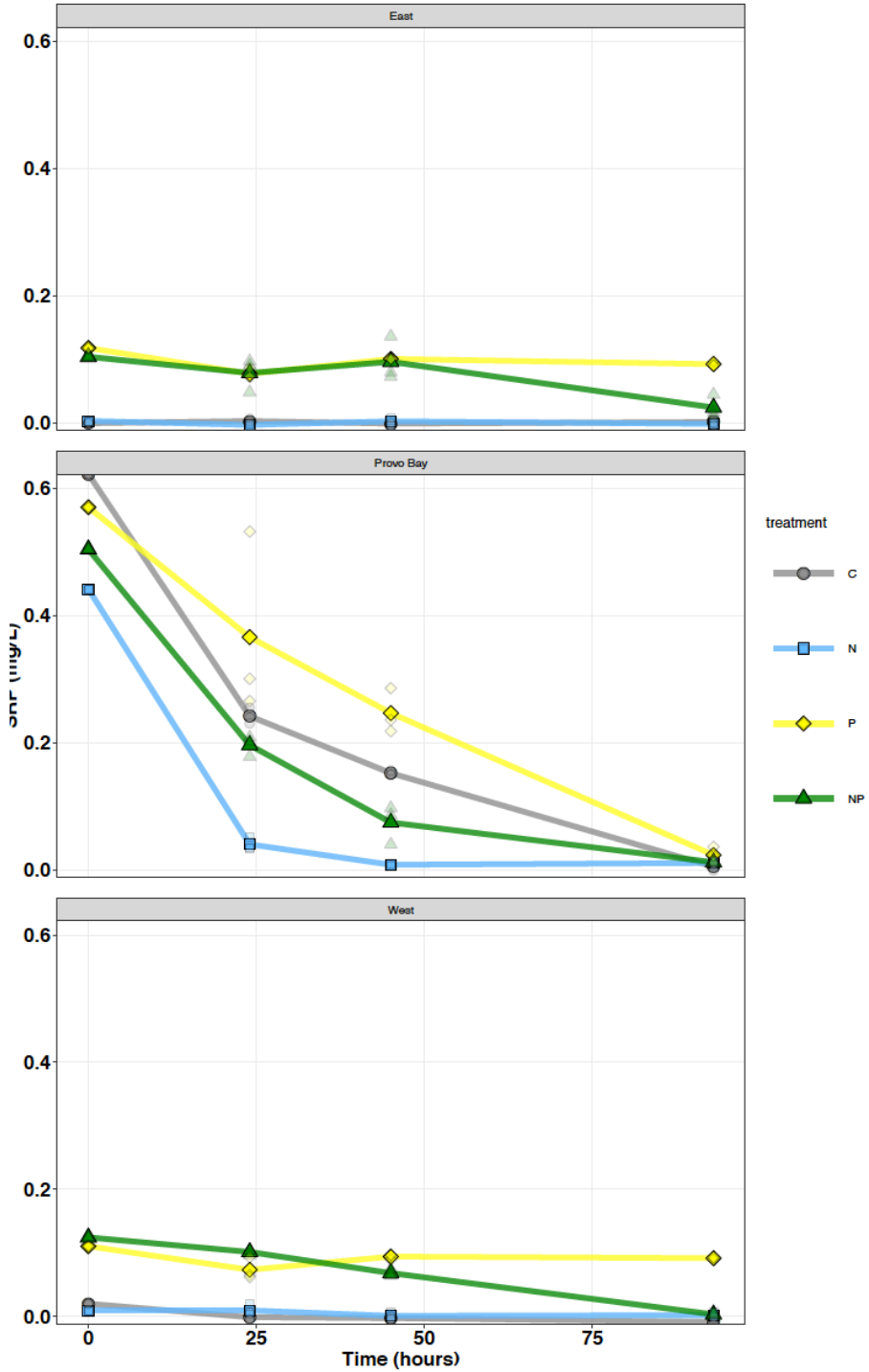
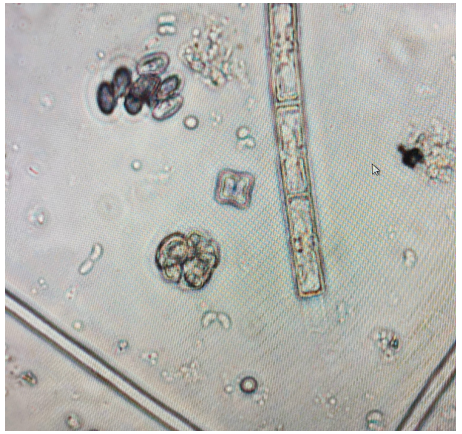


Fig. 8. Threshold relationship between DIN (the sum of NO_3-N and NH_4-N) and the nitrogen **(N)** addition ΔR limitation response ($\log_{10}$ ratio of nutrient treatment growth relative to the control per day) for the total phytoplankton assemblage, chlorophytes, cyanobacteria, and diatoms (**a**). Threshold relationship between the DIN to SRP ratio and the nitrogen (N) addition ΔR limitation response (**b**). Threshold relationship between SRP concentration and the P addition ΔR limitation response (**c**), and the threshold relationship between the DIN:SRP ratio and the P addition ΔR limitation response (**d**). Note: Solid vertical lines indicate statistical significance ($\alpha < 0.05$), and dashed vertical lines indicate marginal significance ($\alpha > 0.05$ and < 0.10).


East
Provo Bay
West
0.6
0.4
0.2
0.0
0
25
50
75
Time (hours)
treatment
C
N
P
NP

We completed bioassays in summer (22-26 July), late summer (26-30 August), and fall (7-11 October) across the three lake locations. To date, we have completed three of the five bioassays. We will add a zooplankton “grazer” removal trial to the spring and nutrient dilution trial to early summer bioassays.



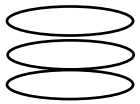
Probe readings
-200 mL rinse
-300 mL for measurement
-poured into EXO storage cup

Microscopy evaluation of Cyanobacteria and algae
-100 mL
-Preserved with 2 mL Lugol's fixing agent

Toxin (ELISA)
-2 60-mL amber vials
One vial for anatoxin A receives 4.5 mL due to added preservative
-Kept on ice

Nutrients and pigments
-1 L
-Kept on ice

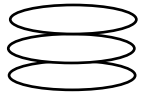
RNA filtering
-1 L
-Kept on ice



TSS/VSS, Chl α, & phycocyanin

Filtered SRP, DIN

Unfiltered TN/TP



Frozen -80°C for future Cyanobacterial biomass estimates via quantitative PCR, bacterial community composition, and *nif*H gene quantification